Do Inhaled Ambient Particles Induce Oxidative Stress and Inflammation in the Brain?

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- The statements and conclusions in this presentation are those of the presenter and not necessarily those of the California Air Resources Board. The mention of commercial products, their source, or their use in connection with material reported herein is not to be construed as actual or implied endorsement of such products.

Environmental Effects on the Brain may be Important

- Degenerative brain disease incidences are increasing and may be irreversible.
- There is increasing evidence for a role of environmental interactions in the rising disease rates.
- Mechanisms are elusive, at best.

Background

- Evidence from epidemiological studies demonstrated that brains of individuals (humans and dogs) living in areas with elevated levels of ambient PM exhibited inflammation and lesions. Subsequent controlled studies have supported some of these early findings.
- Growing evidence that PM exposure increases production of inflammatory mediators and damages or kills brain cells.
- PM exposure can affect cells that are essential for the production and metabolism of the neurotransmitter dopamine.

“Morphometric analysis of the CNS indicated unequivocally that the brain is a critical target for PM exposure and implicated oxidative stress as a predisposing factor that links PM exposure and susceptibility to neurodegeneration. Together, these data present evidence for potential translocation of ambient particles on organs distant from the lung and the neurodegenerative consequences of exposure to air pollutants.” (Peters et al. 2006).

Examining environmental effects in the brain is difficult

- Environmental exposures are low so we would need to :
  - Expose for very long times
  - Use very large numbers of test subjects
- But we can engineer the experiment to be more manageable!!
- And by examining brains from mice exposed in different locations with the same experimental design we can possibly dissect out critical sources or components.

Study Objectives

- Determine how the biological responses in the brains of mice exposed to fine PM might depend on the composition of ambient PM emitted from vehicles, power generation, industrial processes and other sources.
- Address the question, “Are PM-induced biochemical changes in the CNS influenced by PM concentration, source or compositional differences?”
- *In vivo* biological responses, which have been identified as biomarkers of CNS exposure or injury, were examined with respect to the physical and chemical composition differences between the PM from five sites with distinctly different ambient PM sources.
- Take advantage of a unique opportunity for resource leveraging because the very costly and involved exposure and atmosphere characterization phases were mostly underwritten by a multimillion dollar HEI grant.

Experimental Design

- Mice were exposed in New York City (NYC), Sterling Forest, NY (SF), Seattle, WA (SEA), East Lansing, MI (MSU) and Irvine, CA (UCI).
- Exposures were 6 hours per day, 4 days per week for 26 weeks (6 months).
- Brains were harvested and analyzed for inflammatory cytokines (Interleukin-6, Interleukin-10 and Tumor Necrosis Factor-α), reduced and oxidized glutathione, as markers for anti-oxidant defenses, and biomarkers of oxidative stress (protein carbonyls, hydroxynonenal and malondialdehyde).
- Particulate samples from the exposure atmosphere were collected and analyzed for mass concentration, elemental and organic carbon contents and trace metal composition.
- The component data were used in a source apportionment analysis to identify the key sources that contributed to the PM2.5 at each of the five locations.

Experimental Design (cont.)

- All animals used in this study were apoE-/- mice and each exposure group consisted of 16 mice.
- Mice were exposed to FA or CAPs for 2 months, 4 months and 6 months. All protocols were approved by Institutional Animal Care and Use Committees.
- Six FA and six Caps mice were implanted with telemetry devices and were exposed for 6 months. The implanted mice were housed singly so that ECG parameters could be monitored while the mice were in the vivarium.
- While in housing the animals breathed filtered, purified air and were provided with food and water *ad libitum*.
- Telemeter-equipped mice were monitored during exposures and while they were in the vivarium, but were not monitored during loading and unloading to the exposure chambers.
- On the average, mice were monitored about 22 hr per day. ECG data from the same time periods were evaluated on exposure and non-exposure days so the unmonitored preparation time did not impact our analyses.

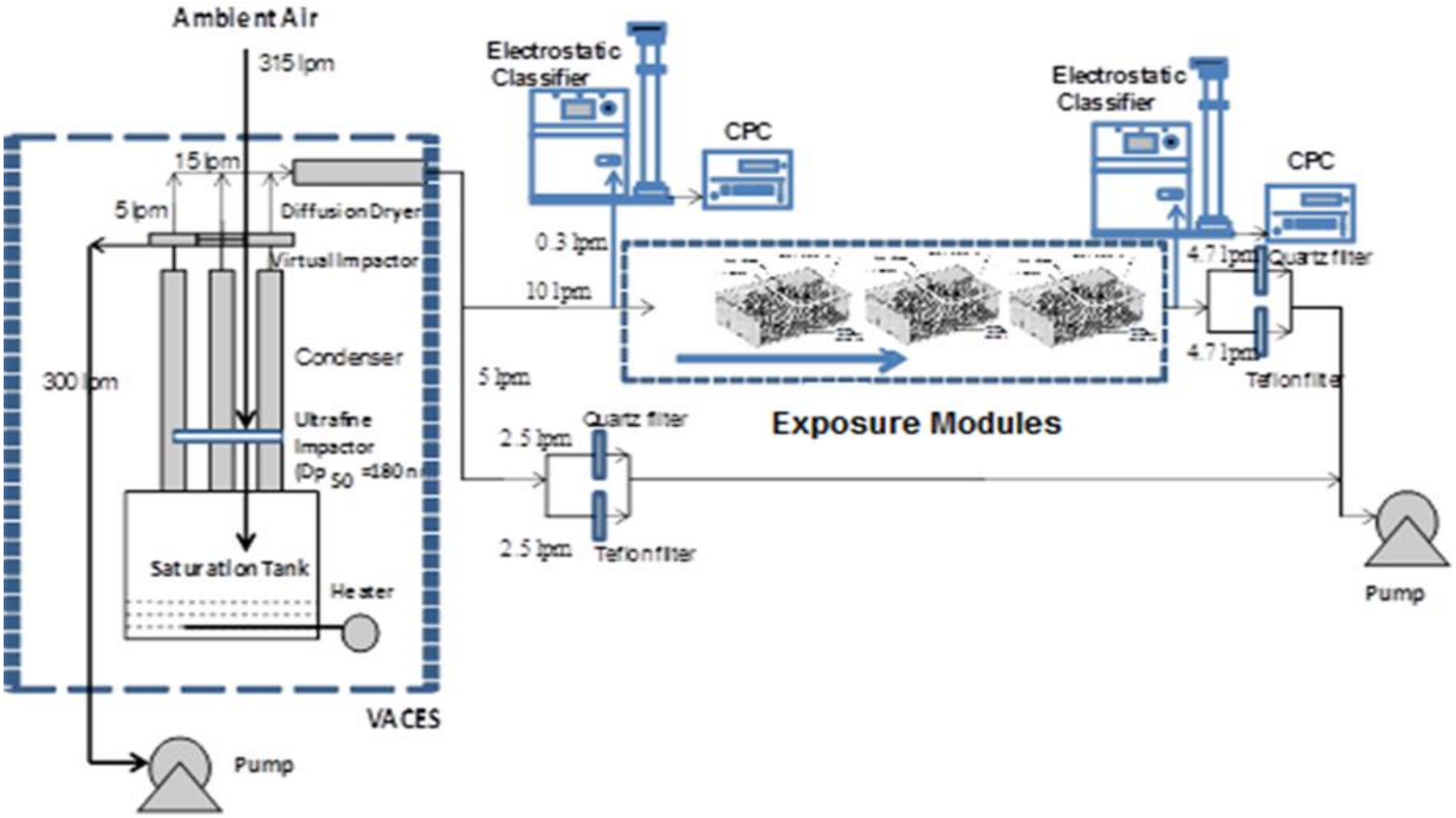
Exposure Site Characteristics

- NYC is a densely populated urban environment with major impacts from traffic and oil-fired power generation.
- SF is a rural location with regional source contributions.
- The SEA location is on a university campus about 8 miles north of downtown Seattle (a site with moderate traffic and a major influence from wood smoke emissions from space heating during the winter).
- MSU is an urban area which is influenced by coal burning sources and is more industrial than the other locations.
- UCI is proximate to heavily trafficked roadways with some influence from regional coastal sources that could include emissions from oil refineries and operations at the Ports of Long Beach and Los Angeles when winds are from the north and west.

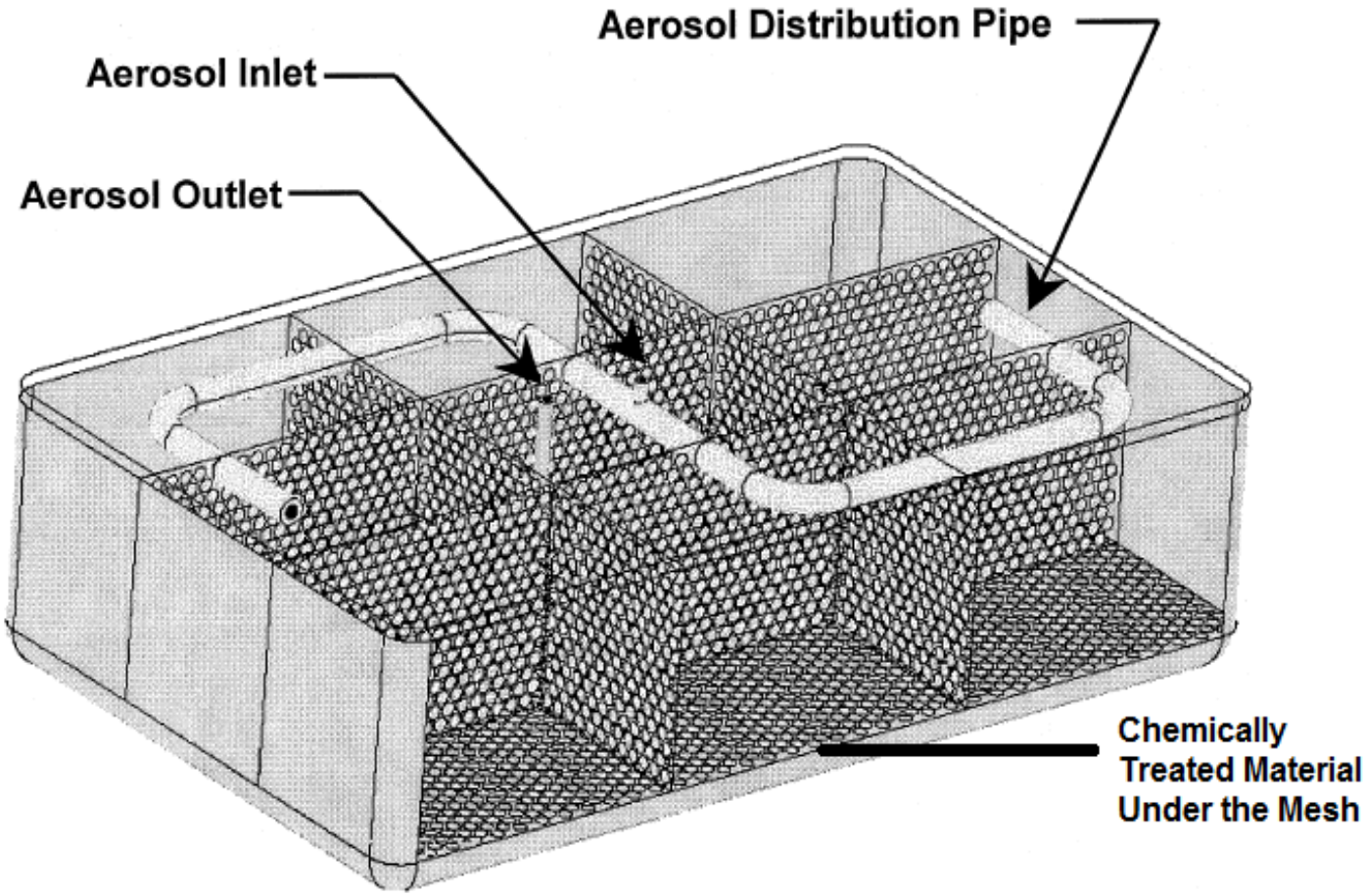
Ambient Particle Concentrator

- Ambient particles with particle diameters smaller than 2.5 µm (PM2.5), including the ultrafine fraction of ambient PM, were concentrated using the Versatile Aerosol Concentration Enrichment System (VACES) which has been described in detail by Kim et al. (Kim et al. 2001a; Kim et al. 2001b).

System for Exposing Mice to Concentrated Ambient Particles (CAPs).



Schematic diagram of the mobile exposure cage.



CAPs Concentrations and Composition Data Averaged For 6 Month Mouse Exposures

	New York, NY 2007		Sterling Forest, NY 2007		Seattle, WA 2009		East Lansing, MI 2010		Irvine, CA 2011	
	Mean	Std	Mean	Std	Mean	Std	Mean	Std	Mean	Std
Ambient	20	13	17	13	7	4	8	6	10	6
CAPs	123	81	136	111	61	36	68	55	138	89
BC	2667	1521	785	514	1028	755	526	311	1422	1189
EC	1206	765	375	277	NA	NA	256	241	710	617
OC	8343	6030	3438	2373	NA	NA	5540	1575	7380	1795

[1] CAPs concentrations are in μg/m^3; component concentrations are in ng/m^3.
[2] BC measured using an Aethalometer during the exposure period (0900-1500); EC/OC measured using a Sunset Lab instrument on daily PM collections on quartz filters
[3] NA Not Analyzed

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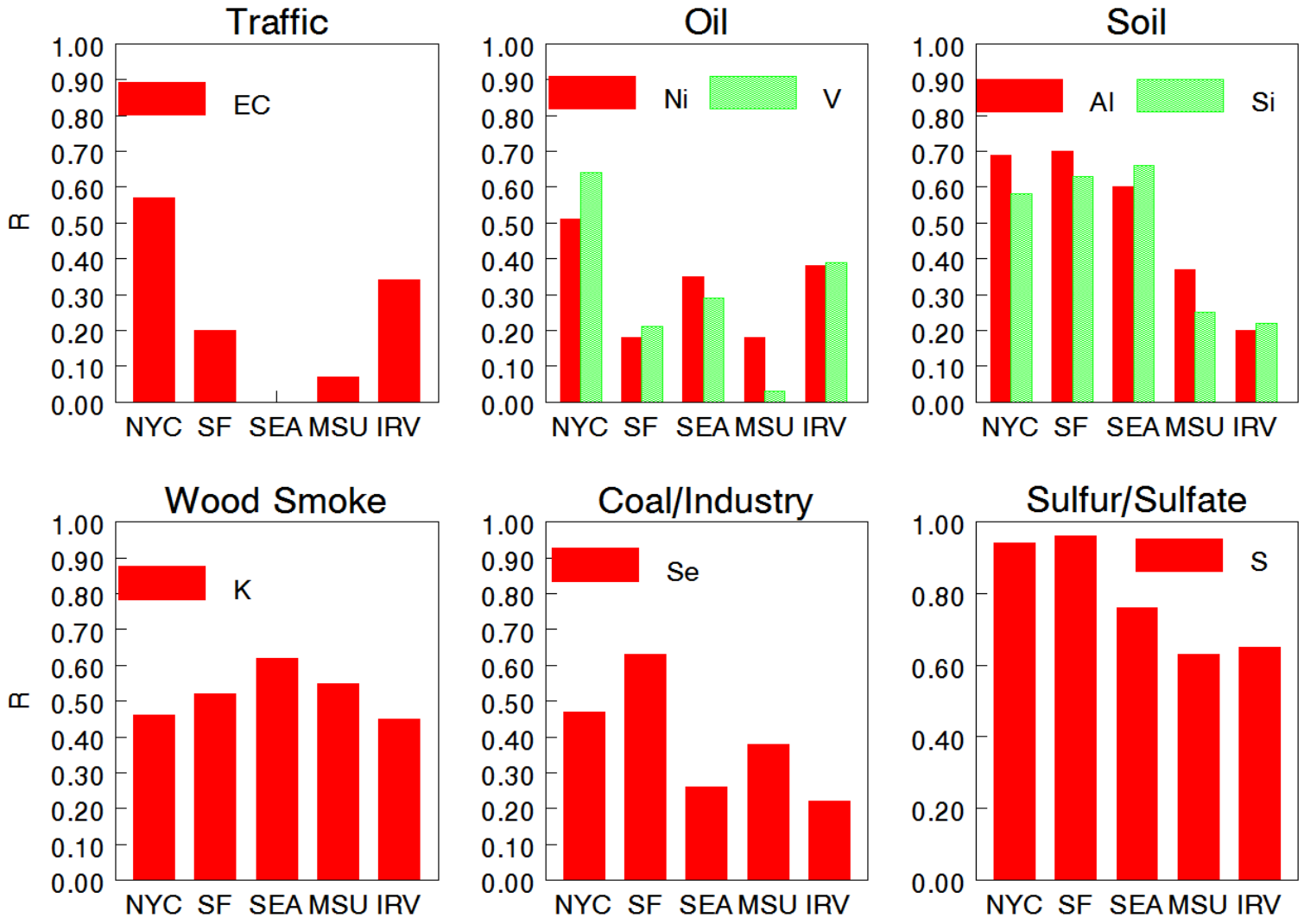
OC	8343	6030	3438	2373	NA	NA	5540	1575	7380	1795
Al	1116	623	931	584	858	466	844	539	1006	608
Br	27	19	27	21	20	12	26	16	65	38
Ca	1204	574	223	148	502	290	436	419	690	453
Cr	16	18	19	21	26	19	9	16	17	12
Cu	63	36	22	19	41	28	5	10	87	108
Fe	1879	888	466	362	921	614	302	210	1610	1538
K	429	363	323	300	376	427	298	249	479	215
Mg	360	252	252	201	495	315	291	245	925	648
Mn	107	75	14	12	31	28	16	16	35	32
Na	845	729	502	587	1733	1596	227	287	5024	4540
Ni	70	62	16	16	18	19	7	7	14	12
P	257	216	219	235	66	68	137	205	142	162
Pb	153	114	55	34	43	23	21	28	25	25
S	11259	10432	17686	19689	3842	2702	6518	7103	7594	5925
Se	9	9	10	12	6	6	20	15	21	13
Si	1658	929	989	798	1399	1005	797	750	1911	1515
Tl	77	45	53	34	49	35	21	18	84	74
V	42	44	17	16	26	35	20	13	46	34
Zn	760	1194	78	97	126	90	51	45	100	92

Site-Specific Source Results

- The relative importance of different sources was examined using the strength of the correlation of selected source tracer elements concentrations to PM concentrations during the 6 months of study at each site.
- Traffic contributions, using EC as a tracer, were important in NYC and UCI exposures. Unfortunately, EC was not measured in SEA. EC is generally associated with the ultrafine size fraction of $PM_{2.5}$.
- The influence of oil combustion using Ni and V as tracers, was highest in NYC but was also substantial for SEA and UCI.
- Soil and crustal influences using Al and Si as tracers were most important for NYC, SF and SEA. These elements are generally associated with particles 2 µm aerodynamic diameter and larger.
- The impact of wood smoke, using K as a tracer, was greatest in SF and SEA but some influence was also seen in UCI. The findings of an influence of wood smoke at UCI probably reflected incursions of wildfire smoke from uncontrolled burns that occurred in 2010, as opposed to the use of wood burning for heating purposes in SF and SEA.
- The impact of coal and industrial emissions using Se as a tracer were greatest in NYC, SF and MSU, all locations with known impacts from coal-burning power plant emissions.

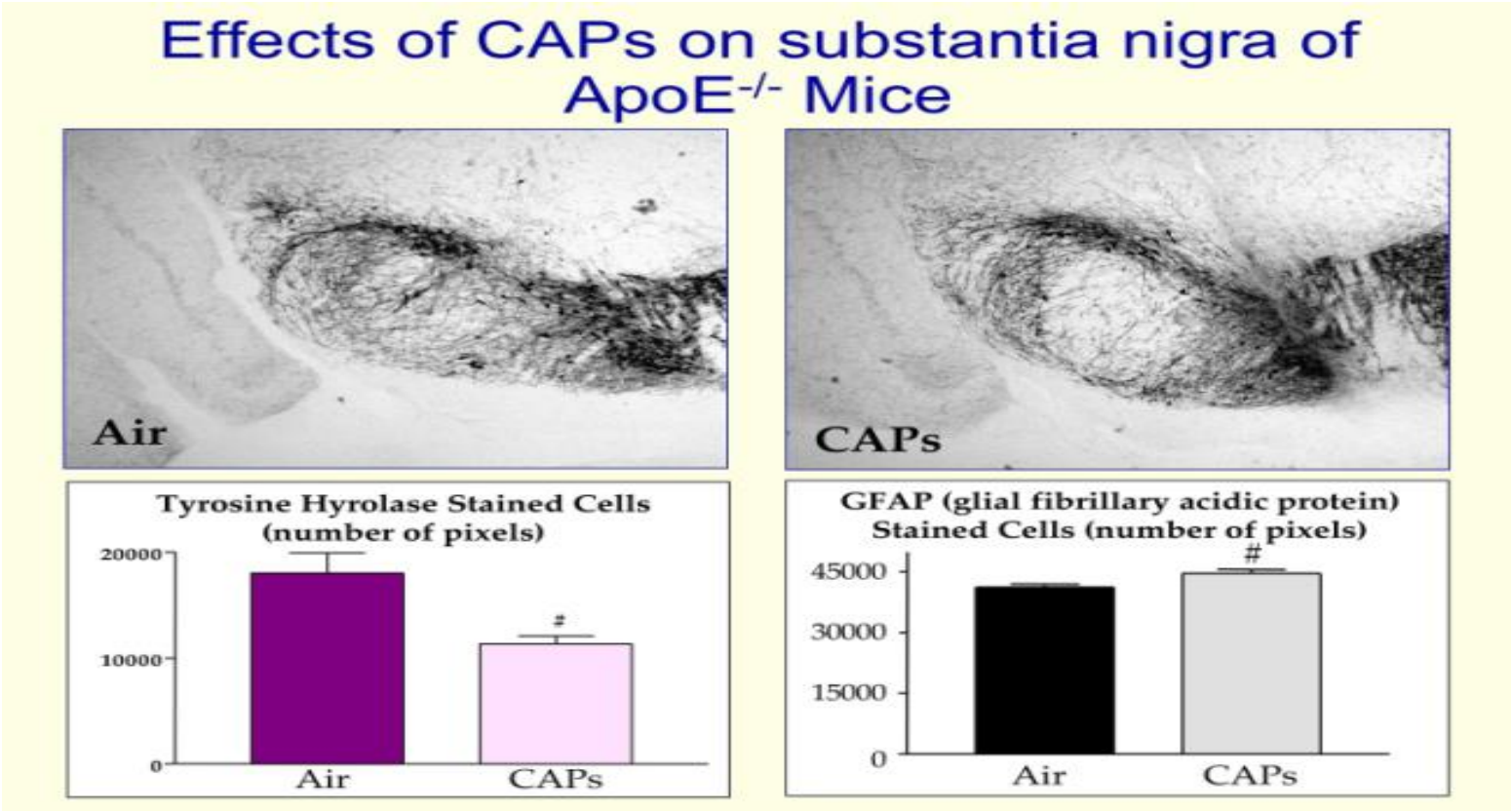
Source Contributions to CAPs Based on a Chemical Mass Balance Tracer Method



Biological Response Data – NYC and SF

- Results were published and have been summarized by MohanKumar and colleagues in a review article (MohanKumar et al. 2008).
- The brains of CAPs-exposed and FA-exposed apoE-/- mice were sectioned iand stained for tyrosine hydroxylase (TH), a marker for dopamine (DA) producing neurons, and for glial fibrillary acidic protein (GFAP), a marker of astrocytic proliferation.
- Statistical analysis of TH stained neurons with their attached fibers showed no significant difference in air or CAPs-exposed normal background control C57/blk6. However, there was a 29% reduction in TH stained neurons in the CAPs exposed brains of apoE-/-mice relative to FA-exposed apoE-/- mice.
- In addition, there was a significant 8% increase ($p<0.05$) in GFAP staining (i.e., astrocytes) in the nucleus compacta of CAPs exposed apoE-/- mice relative to FA-exposed apoE-/- mouse brains.
- Concentrations of inflammatory cytokines or markers of oxidative stress were not measured in the brains of these mice.

Inhalation of fine and ultrafine particles injures or kills cells in the brain that make dopamine from tyrosine hydroxylase in the region called the substantia nigra. This process may be caused by activation of immune system cells that are identified using a stain for glial fibrillary acidic protein (GFAP) [2]



2. Veronesi et al., Inhal Toxicol 17: 235-41; 2005.

Biological Response Data – UCI, SEA and MSU Sites

- The samples from UCI were sectioned into rostral, mid and caudal fractions .
- Regional inflammatory and oxidative-stress related responses, how those responses were differentially expressed as a function of length of exposure (2 months vs. 4 months vs. 6 months of exposure) were examined

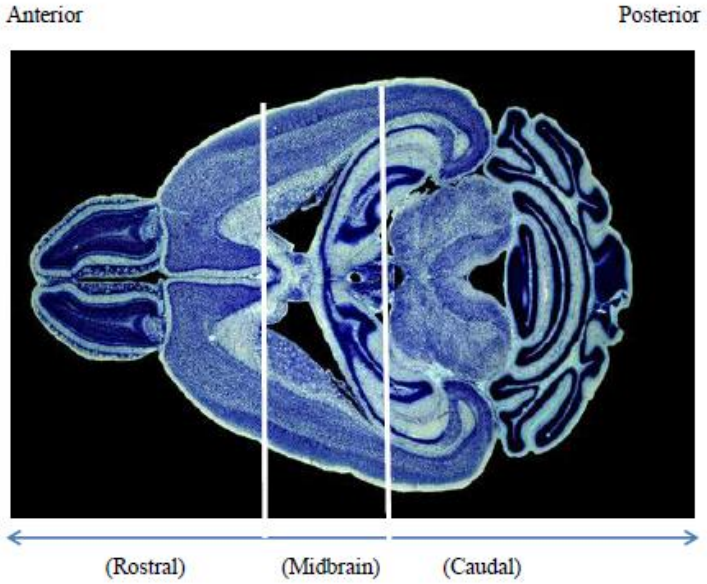


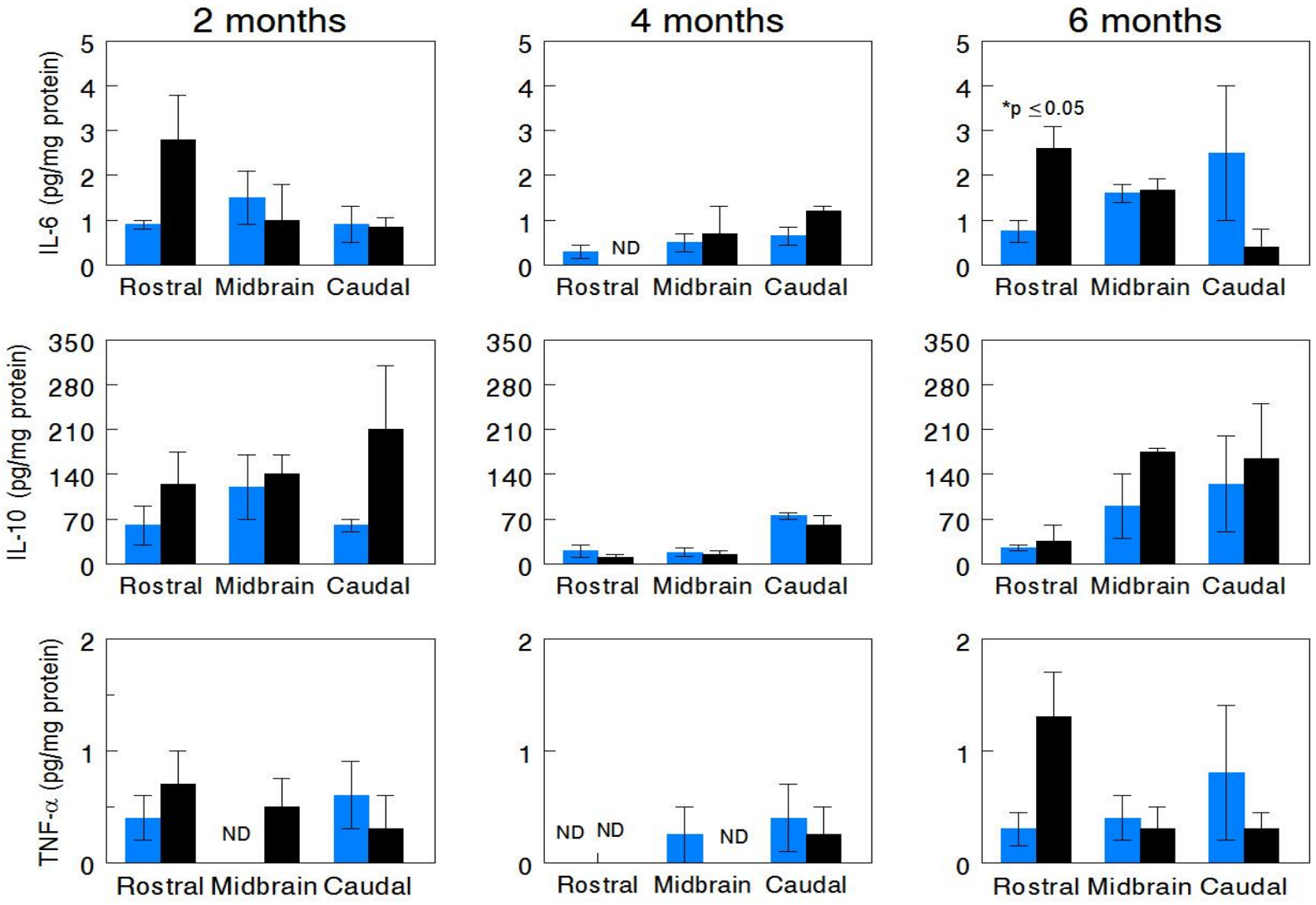
Figure 4. Horizontal section through a mouse brain (adapted from Mouse Brain Atlas - http://www.mbl.org/atlas005/atlas005_frame.html).

Inflammatory cytokines (IL-6, IL-10 and TNF-α) in cytoplasmic fractions of brain sections

- None of the three cytokines demonstrated a consistent trend with time, i.e. average levels across the 3 regions of the brain were not significantly different at 2 months vs. 6 months exposure, although the concentrations for all three cytokines were lower at 4 months than after 2 or 6 months of exposures.
- There were significant regional differences at 6 months. IL-6 ($p \leq 0.05$) and TNF-α ($p \leq 0.10$) concentrations were increased in the rostral sections of the brains in CAPs-exposed mice compared to the levels in the FA-exposed mice.

Inflammatory cytokine levels in cytoplasmic fraction of brain homogenates

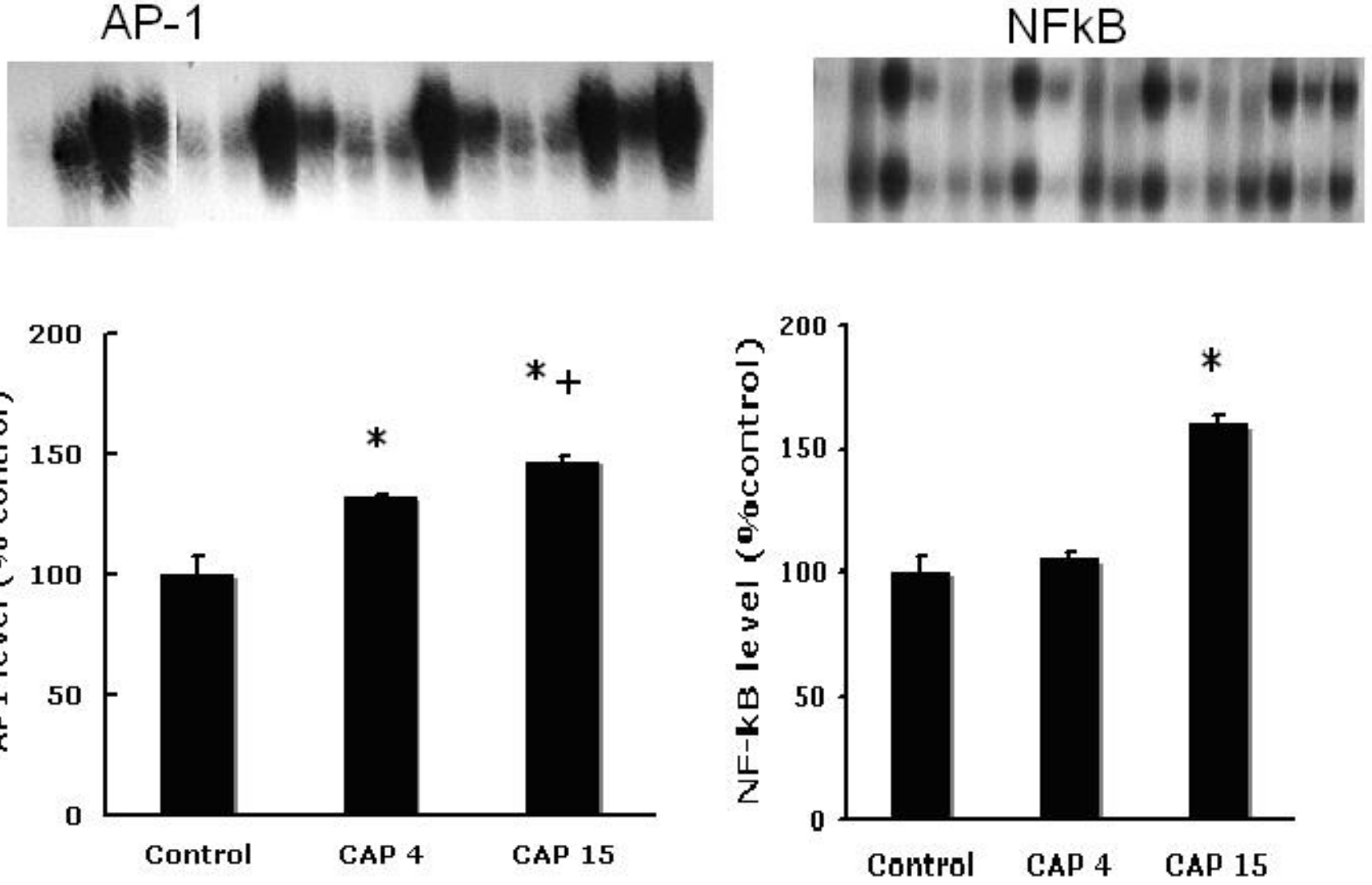
BLUE = FA; BLACK = CAPs (n=4 for each time point)



One Possible Mechanism for Induction of Inflammation by Quasi-Ultrafine Particle Exposure

- The concentrations of Nf-κB and AP-1, two transcription factors that control gene expression of proteins related to inflammatory responses, were increased in the brains of mice by both low (CAP 4 = ~ 30 μg/m^3) and high (CAP 15 = ~100 μg/m^3) concentrations of quasi-ultrafine PM.
- There were some changes in the expression of mitogen activated protein kinases (MAPK) that regulate various other transcription factors in the brain but a clear pattern has not yet emerged.
- Activated NFkB translocates to the cell nucleus and induces the expression of inflammatory cytokines including TNFα and IL-1

Ultrafine Particle Exposure Increases Expression of AP-1 and NFkB

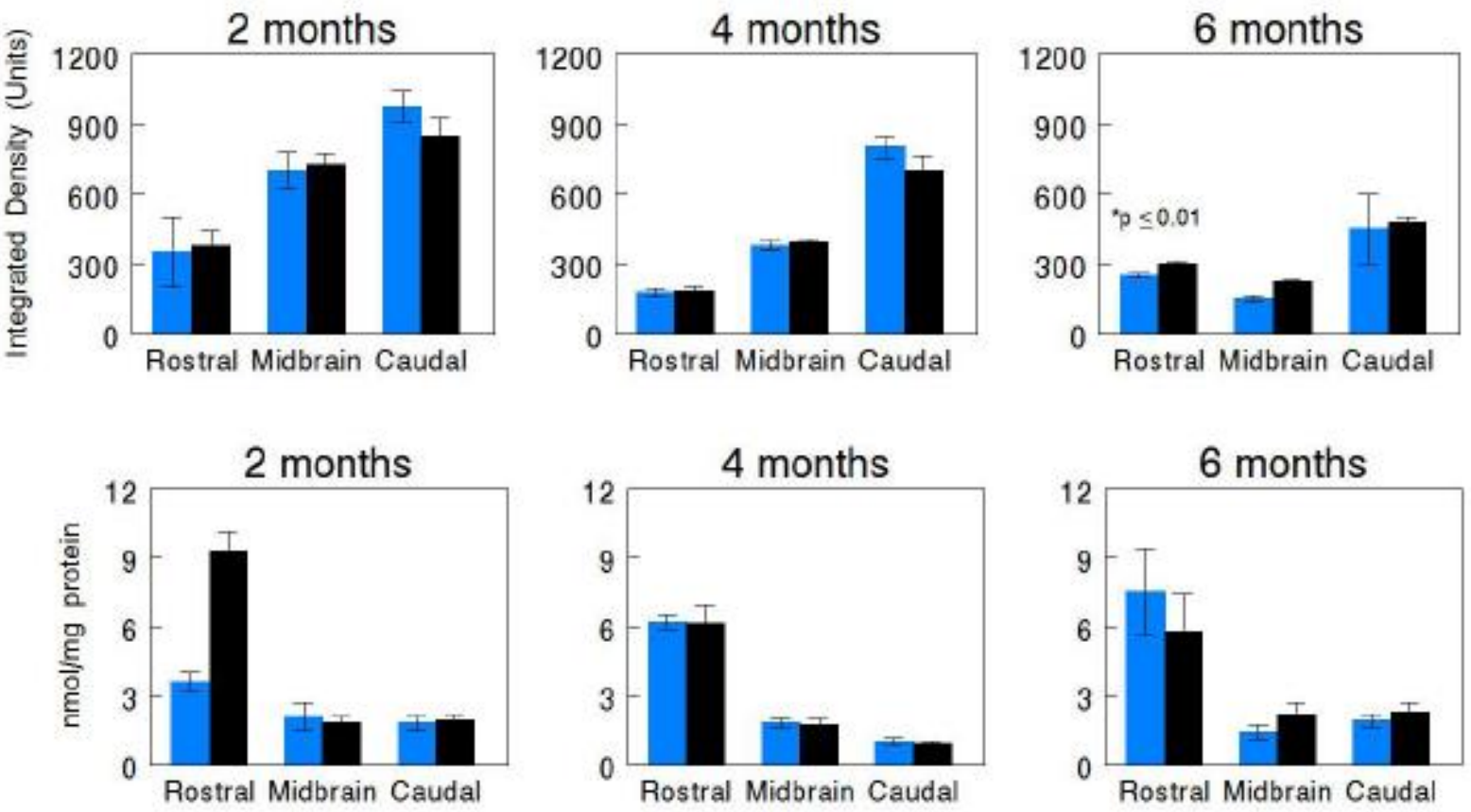


Biochemical marker levels in nuclear fraction of brain homogenates of UCI mice

- There was an increase in NFκB activity in the rostral sections of mice exposed to CAPs at all three time points, which achieved significance after 6 months of exposure ($p \leq 0.01$).
- This is consistent with the increased levels of IL-6 and TNFα in rostral sections of the brains of CAPs-exposed mice shown in the previous slide.
- Protein Carbonyl levels showed a regional pattern inversely related to that of NFkB but did not evidence an exposure-related effect.

NF-κB and Protein Carbonyl levels in nuclear fraction of brain homogenates in UCI mice

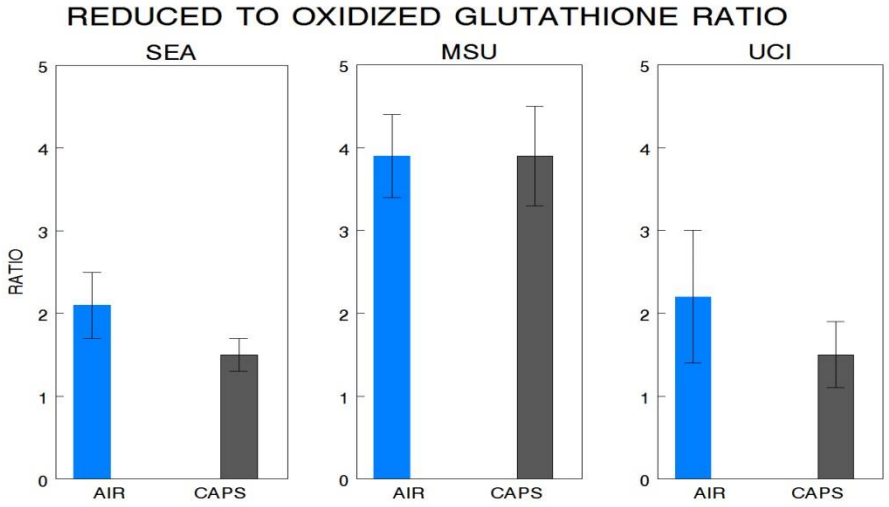
BLUE = FA; BLACK = CAPs, n=4 per exposure

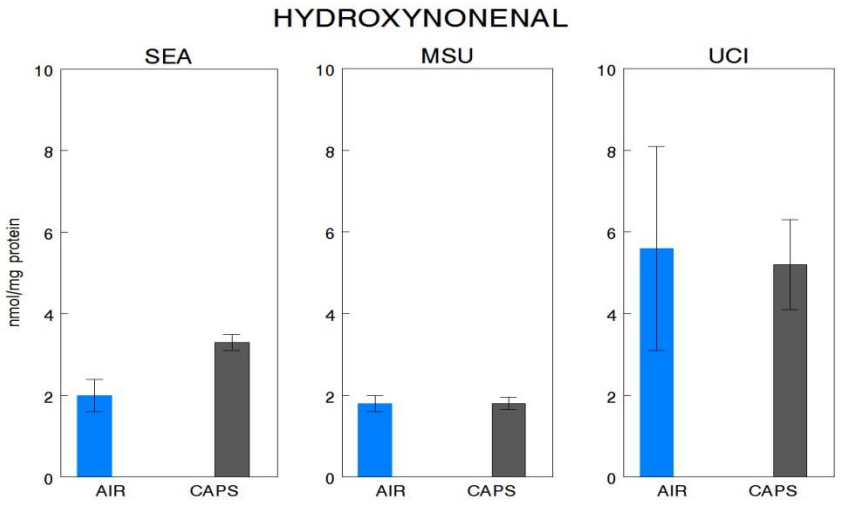


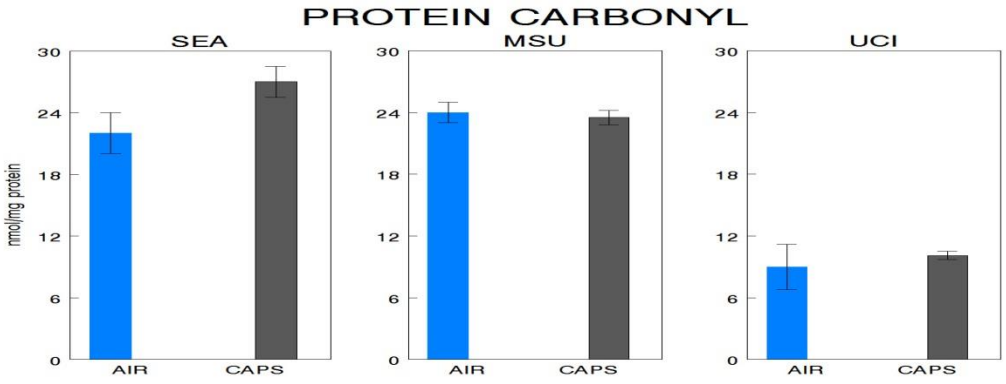
Exposure Site-Specific Differences in Brain Response to CAPs Exposure at SEA, MSU and UCI

- The ratio of reduced (GSH) to oxidized (GSSG) glutathione is a sensitive indicator of how exposure can affect the anti-oxidant capacity of cells or tissues.
- Levels of the active (*i.e.* reduced) glutathione appeared slightly reduced after 6 month exposures at the SEA ($p \leq 0.10$) and UCI (n.s.) but were not changed in the brains of mice exposed at MSU.
- Hydroxynonenal levels (were significantly increased in the brains of mice exposed at SEA but there were no differences observed in mice exposed at either MSU or UCI.
- MDA in the brains of CAPs-exposed mice were lower and the levels of hydroxynonenal were about the same or slightly increased (as compared with brains from air-exposed mice) which is an unusual and at this time unexplained occurrence.
- Protein carbonyl concentrations were increased in whole brain samples of mice exposed to CAPs in SEA ($p \leq 0.05$) and UCI (not significant).

Oxidant Defenses and Pro-Oxidant Effects BLUE=FA; BLACK=CAPs, n=12







Conclusions

- Exposure of apoE-/- mice to CAPs was associated with inflammatory changes in the brain, and on a regional basis, the sections of the brain that were lower in the signal transducer nuclear factor-κB (NF-κB) tended to be more susceptible to inflammatory changes.
- The levels of NF-κB decreased as the animals aged during the 6 month study in both Filtered Air (FA) and CAPs-exposed mice. Sections of the brain with lower NF-κB levels also tended to exhibit exposure-related increases in concentrations of biomarkers of oxidative stress, consistent with a correlation between inflammation and oxidative changes in the brain.
- Oxidative changes in the brain were consistently observed in mice exposed at SEA but not in mice exposed at MSU.
- Mice exposed at UCI showed a pattern of changes that was the same as that seen in SEA mice however because the UCI brains had been sectioned for brain regional analysis, the variances were larger in the UCI group than in the SEA group and the average effect differences compared to FA-exposed brains did not achieve statistical significance.

Conclusions, cont.

- The sources of PM at the UCI and SEA sites were more influenced by emissions related to oil combustion (such as motor vehicles, power generation, space heating) than was the PM at the MSU site, as evidenced by a high correlation of PM concentrations with the concentrations of Ni and V in the particles at UCI and SEA but not at MSU.
- The concentrations of Ni and V in the exposure atmosphere were probably too low to be directly toxic and it is likely that these elements are tracers or surrogates for oil combustion aerosols which would include EC, OC and BC.
- The pattern of more exposure-related increases in oxidative stress markers at SEA and UCI relative to MSU could indicate that the products of oil combustion from mobile, power generation and space heating sources may be important factors in the inflammatory and oxidative changes noted in the brains of apoE-/- mice exposed to CAPs.

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Moving the AMS is a group effort!

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